STREPTOCOCCUS PYOGENES POLYPEPTIDES AND CORRESPONDING DNA FRAGMENTS

FIELD OF THE INVENTION

The present invention is related to polypeptides of Streptococcus pyogenes (Group A Streptococcus) which may be used to prevent, diagnose and/or treat streptococcal infection.

BACKGROUND OF THE INVENTION

Streptococci are gram (+) bacteria which are differentiated by group specific carbohydrate antigens A through O which are found at the cell surface. S. pyogenes isolates are further distinguished by type-specific M protein antigens. M proteins are important virulence factors which are highly variable both in molecular weights and in sequences. Indeed, more than 80-M protein types have been identified on the basis of antigenic differences.

S. pyogenes is responsible for many diverse infection types, including pharyngitis, erysipelas and impetigo, scarlet fever, and invasive diseases such as bacteremia and necrotizing fasciitis. A resurgence of invasive disease in recent years has been documented in many countries, including those in North America and Europe. Although the organism is sensitive to antibiotics, the high attack rate and rapid onset of sepsis results in high morbidity and mortality.

To develop a vaccine that will protect hosts from S. pyogenes infection, efforts have focused on virulence factors such as the type-specific M proteins. However, the amino-terminal portion of M proteins was found to induce cross-reactive antibodies which reacted with human myocardium, tropomyosin, myosin, and vimentin, which might be implicated in autoimmune diseases. Others have used recombinant techniques to produce complex hybrid proteins containing amino-terminal peptides of M proteins from different

serotypes. However, a safe vaccine containing all S. pyogenes serotypes will be highly complex to produce and standardize.

In addition to the serotype-specific antigens, other S. pyogenes proteins have generated interest as potential vaccine candidates. The C5a peptidase, which is expressed by at least S. pyogenes 40 serotypes, was shown to be immunogenic in mice, but its capacity to reduce the level of nasopharyngeal colonization was limited. Other investigators have also focused on the streptococcal pyrogenic exotoxins which appear to play an important role in pathogenesis of infection. Immunization with these proteins prevented the deadly symptoms of toxic shock, but did not prevent colonization.

The University of Oklahoma has set up a genome sequencing project for S. pyogenes strain M1 GAS (http://dna1.chem.ou.edu/strep.html).

Therefore there remains an unmet need for S. pyogenes antigens that may be used vaccine components for the prophylaxis and/or therapy of S. pyogenes infection.

SUMMARY OF THE INVENTION

According to one aspect, the present invention provides an isolated polynucleotide encoding a polypeptide having at least 70% identity to a second polypeptide comprising SEQ ID No : 2 or fragments or analogs thereof.

According to one aspect, the present invention relates to polypeptides which comprise an amino acid sequence SEQ ID No : 2 or fragments or analogs thereof.

In other aspects, there are provided polypeptides encoded by polynucleotides of the invention, pharmaceutical compositions, vectors comprising polynucleotides of the invention operably linked to an expression control region, as well as host cells transfected with said vectors and processes for producing polypeptides

comprising culturing said host cells under conditions suitable for expression.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 represents the DNA sequence of BVH-P7 gene from serotype M1 S. pyogenes strain ATCC700294; SEQ ID NO: 1. The underlined portion of the sequence represents the region coding for the leader peptide.

Figure 2 represents the amino acid sequence BVH-P7 protein from serotype M1 S. pyogenes strain ATCC700294; SEQ ID NO: 2. The underline sequence represents the 21 amino acid residues leader peptide.

Figure 3 depicts the comparison of the predicted amino acid sequences of the BVH-P7 open reading frames from Spy74, Spy70, Spy69, Spy68, Spy 60, ATCC12357, ATCC700294 S. pyogenes strains by using the program Clustal W from MacVector sequence analysis software (version 6.5). Underneath the alignment, there is a consensus line where * and . characters indicate identical and similar amino acid residues, respectively.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides purified and isolated polynucleotides, which encode Streptococcal polypeptides that may be used to diagnose, prevent, and/or treat Streptococcal infection.

According to one aspect, the present invention provides an isolated polynucleotide encoding a polypeptide having at least 70% identity to a second polypeptide comprising SEQ ID NO: 2 or fragments or analogs or thereof.

According to one aspect, the present invention provides an isolated polynucleotide encoding a polypeptide having at least 80% identity to a second polypeptide comprising SEQ ID NO: 2 or fragments or analogs or thereof.

According to one aspect, the present invention provides an isolated polynucleotide encoding a polypeptide having at least 90% identity to a second polypeptide comprising SEQ ID NO: 2 or fragments or analogs or thereof.

According to one aspect, the present invention provides an isolated polynucleotide encoding a polypeptide having at least 95% identity to a second polypeptide comprising SEQ ID NO: 2 or fragments or analogs or thereof.

According to one aspect, the present invention relates to polypeptides characterized by the amino acid sequence comprising SEQ ID NO: 2 or fragments or analogs or thereof.

According to one aspect, the present invention provides a polynucleotide encoding an epitope bearing portion of a polypeptide comprising SEQ ID NO: 2 or fragments or analogs or thereof.

According to one aspect, the present invention relates to epitope bearing portions of a polypeptide comprising SEQ ID NO: 2 or fragments or analogs or thereof.

According to one aspect, the present invention provides an isolated polynucleotide encoding a polypeptide having at least 70% identity to a second polypeptide comprising SEQ ID NO: 2.

According to one aspect, the present invention provides an isolated polynucleotide encoding a polypeptide having at least 80% identity to a second polypeptide comprising SEQ ID NO: 2.

According to one aspect, the present invention provides an isolated polynucleotide encoding a polypeptide having at least 90% identity to a second polypeptide comprising SEQ ID NO: 2.

According to one aspect, the present invention provides an isolated polynucleotide encoding a polypeptide having at least 95% identity to a second polypeptide comprising SEQ ID NO: 2.

According to one aspect, the present invention relates to polypeptides characterized by the amino acid sequence comprising SEQ ID NO: 2.

According to one aspect, the present invention provides a polynucleotide encoding an epitope bearing portion of a polypeptide comprising SEQ ID NO: 2.

According to one aspect, the present invention relates to epitope bearing portions of a polypeptide comprising SEQ ID NO: 2.

According to one aspect, the present invention provides an isolated polynucleotide comprising a polynucleotide chosen from:

(a) a polynucleotide encoding a polypeptide having at least 70% identity to a second polypeptide comprising a sequence chosen from: SEQ ID NO: 2 or fragments or analogs thereof;

(b) a polynucleotide encoding a polypeptide having at least 95% identity to a second polypeptide comprising a sequence chosen from: SEQ ID NO: 2 or fragments or analogs thereof;

(c) a polynucleotide encoding a polypeptide comprising a sequence chosen from: SEQ ID NO: 2 or fragments or analogs thereof;

(d) a polynucleotide encoding a polypeptide capable of generating antibodies having binding specificity for a polypeptide comprising a sequence chosen from: SEQ ID NO: 2 or fragments or analogs thereof;

(e) a polynucleotide encoding an epitope bearing portion of a polypeptide comprising a sequence chosen from SEQ ID NO: 2 or fragments or analogs thereof;

(f) a polynucleotide comprising a sequence chosen from SEQ ID NO: 1 or fragments or analogs thereof;

(g) a polynucleotide that is complementary to a polynucleotide in (a), (b), (c), (d), (e) or (f).

According to one aspect, the present invention provides an isolated polynucleotide comprising a polynucleotide chosen from:

(a) a polynucleotide encoding a polypeptide having at least 70% identity to a second polypeptide comprising a sequence chosen from: SEQ ID NO: 2;

(b) a polynucleotide encoding a polypeptide having at least 95% identity to a second polypeptide comprising a sequence chosen from: SEQ ID NO: 2;

(c) a polynucleotide encoding a polypeptide comprising a sequence chosen from: SEQ ID NO: 2;

(d) a polynucleotide encoding a polypeptide capable of raising antibodies having binding specificity for a polypeptide comprising a sequence chosen from: SEQ ID NO: 2;

(e) a polynucleotide encoding an epitope bearing portion of a polypeptide comprising a sequence chosen from SEQ ID NO: 2;

(f) a polynucleotide comprising a sequence chosen from SEQ ID NO: 1;

(g) a polynucleotide that is complementary to a polynucleotide in (a), (b), (c), (d), (e) or (f).

According to one aspect, the present invention provides an isolated polypeptide comprising a polypeptide chosen from:

(a) a polypeptide having at least 70% identity to a second polypeptide comprising SEQ ID NO: 2, or fragments or analogs thereof;

(b) a polypeptide having at least 95% identity to a second polypeptide comprising SEQ ID NO: 2, or fragments or analogs thereof;

(c) a polypeptide comprising SEQ ID NO: 2, or fragments or analogs thereof;

(d) a polypeptide capable of raising antibodies having binding specificity for a polypeptide comprising SEQ ID NO: 2, or fragments or analogs thereof;

(e) an epitope bearing portion of a polypeptide comprising SEQ ID NO: 2, or fragments or analogs thereof;

(f) the polypeptide of (a), (b), (c), (d), (e) or (f) wherein the N-terminal Met residue is deleted;

(g) the polypeptide of (a), (b), (c), (d), (e) or (f) wherein the secretory amino acid sequence is deleted.

According to one aspect, the present invention provides an isolated polypeptide comprising a polypeptide chosen from:

(a) a polypeptide having at least 70% identity to a second polypeptide comprising SEQ ID NO: 2;

(b) a polypeptide having at least 95% identity to a second polypeptide comprising SEQ ID NO: 2;

(c) a polypeptide comprising SEQ ID NO: 2;

(d) a polypeptide capable of raising antibodies having binding specificity for a polypeptide comprising SEQ ID NO: 2;

(e) an epitope bearing portion of a polypeptide comprising SEQ ID NO: 2;

(f) the polypeptide of (a), (b), (c), (d), (e) or (f) wherein the N-terminal Met residue is deleted;

(g) the polypeptide of (a), (b), (c), (d), (e) or (f) wherein the secretory amino acid sequence is deleted.

Those skilled in the art will appreciate that the invention includes DNA molecules, i.e. polynucleotides and their complementary sequences that encode analogs such as mutants, variants, homologues and derivatives of such polypeptides, as described herein in the present patent application. The invention also includes RNA molecules corresponding to the DNA molecules of the invention. In addition to the DNA and RNA molecules, the invention includes the corresponding polypeptides and monospecific antibodies that specifically bind to such polypeptides.

In a further embodiment, the polypeptides in accordance with the present invention are antigenic.

In a further embodiment, the polypeptides in accordance with the present invention are immunogenic.

In a further embodiment, the polypeptides in accordance with the present invention can elicit an immune response in a host.

In a further embodiment, the present invention also relates to polypeptides which are able to raise antibodies having binding specificity to the polypeptides of the present invention as defined above.

An antibody that "has binding specificity" is an antibody that recognizes and binds the selected polypeptide but which does not substantially recognize and bind other molecules in a sample, e.g., a biological sample. Specific binding can be measured using an ELISA assay in which the selected polypeptide is used as an antigen.

In accordance with the present invention, "protection" in the biological studies is defined by a significant increase in the survival curve, rate or period. Statistical analysis using the Log rank test to compare survival curves, and Fisher exact test to compare survival rates and numbers of days to death, respectively, might be useful to calculate P values and determine whether the difference between the two groups is statistically significant. P values of 0.05 are regarded as not significant.

In an additional aspect of the invention there are provided antigenic/immunogenic fragments of the polypeptides of the invention, or of analogs thereof.

The fragments of the present invention should include one or more such epitopic regions or be sufficiently similar to such regions to retain their antigenic/immunogenic properties. Thus, for fragments according to the present invention the degree of identity is perhaps irrelevant, since they may be 100% identical to a particular part of a polypeptide or analog thereof as described herein. The present invention further provides fragments having at least 10 contiguous amino acid residues from the polypeptide sequences of the present invention. In one embodiment, at least 15 contiguous amino acid residues. In one embodiment, at least 20 contiguous amino acid residues.

The key issue, once again, is that the fragment retains the antigenic/immunogenic properties.

The skilled person will appreciate that analogs of the polypeptides of the invention will also find use in the context of the present invention, i.e. as antigenic/immunogenic material. Thus, for instance proteins or polypeptides which include one or more additions, deletions, substitutions or the like are encompassed by the present invention.

As used herein, "fragments", "analogs" or "derivatives" of the polypeptides of the invention include those polypeptides in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably conserved) and which may be natural or unnatural. In one embodiment, derivatives and analogs of polypeptides of the invention will have about 70% identity with those sequences illustrated in the figures or fragments thereof. That is, 70% of the residues are the same. In a further embodiment, polypeptides will have greater than 80% identity. In a further embodiment, polypeptides will have greater than 85% identity. In a further embodiment, polypeptides will have greater than 90% identity. In a further embodiment, polypeptides will have greater than 95% identity. In a further embodiment, polypeptides will have greater than 99% identity. In a further embodiment, analogs of polypeptides of the invention will have fewer than about 20 amino acid residue substitutions, modifications or deletions and more preferably less than 10.

These substitutions are those having a minimal influence on the secondary structure and hydropathic nature of the polypeptide. Preferred substitutions are those known in the art as conserved, i.e. the substituted residues share physical or chemical properties such as hydrophobicity, size, charge or functional groups. These include substitutions such as those described by Dayhoff, M. in Atlas of Protein Sequence and Structure 5, 1978 and by Argos, P. in

EMBO J. 8, 779-785, 1989. For example, amino acids, either natural or unnatural, belonging to one of the following groups represent conservative changes:

ala, pro, gly, gln, asn, ser, thr, val;

cys, ser, tyr, thr;

val, ile, leu, met, ala, phe;

lys, arg, orn, his;

and phe, tyr, trp, his.

The preferred substitutions also include substitutions of D-enantiomers for the corresponding L-amino acids.

In an alternative approach, the analogs of the polypeptides of the invention comprise the substitutions disclosed in Figure 3.

In an alternative approach, the analogs could be fusion proteins, incorporating moieties which render purification easier, for example by effectively tagging the desired polypeptide. It may be necessary to remove the "tag" or it may be the case that the fusion polypeptide itself retains sufficient antigenicity to be useful.

The percentage of homology is defined as the sum of the percentage of identity plus the percentage of similarity or conservation of amino acid type.

In one embodiment, analogs of polypeptides of the invention will have about 70% identity with those sequences illustrated in the figures or fragments thereof. That is, 70% of the residues are the same. In a further embodiment, polypeptides will have greater than 75% homology. In a further embodiment, polypeptides will have greater than 80% homology. In a further embodiment, polypeptides will have greater than 85% homology. In a further embodiment, polypeptides will have greater than 90% homology. In a further embodiment, polypeptides will have greater than 95% homology. In a further embodiment, polypeptides will have greater than 99% homology. In a further embodiment, analogs of polypeptides of the invention will have fewer than about 20 amino acid residue

substitutions, modifications or deletions and more preferably less than 10.

One can use a program such as the CLUSTAL program to compare amino acid sequences. This program compares amino acid sequences and finds the optimal alignment by inserting spaces in either sequence as appropriate. It is possible to calculate amino acid identity or similarity (identity plus conservation of amino acid type) for an optimal alignment. A program like BLASTx will align the longest stretch of similar sequences and assign a value to the fit. It is thus possible to obtain a comparison where several regions of similarity are found, each having a different score. Both types of identity analysis are contemplated in the present invention.

In an alternative approach, the analogs or derivatives could be fusion polypeptides, incorporating moieties which render purification easier, for example by effectively tagging the desired protein or polypeptide, it may be necessary to remove the "tag" or it may be the case that the fusion polypeptide itself retains sufficient antigenicity to be useful.

It is well known that is possible to screen an antigenic polypeptide to identify epitopic regions, i.e. those regions which are responsible for the polypeptide's antigenicity or immunogenicity. Methods for carrying out such screening are well known in the art. Thus, the fragments of the present invention should include one or more such epitopic regions or be sufficiently similar to such regions to retain their antigenic/immunogenic properties.

Thus, for fragments according to the present invention the degree of identity is perhaps irrelevant, since they may be 100% identical to a particular part of a polypeptide, analog as described herein.

Thus, what is important for analogs, derivatives and fragments is that they possess at least a degree of the

antigenicity/immunogenicity of the protein or polypeptide from which they are derived.

Also included are polypeptides which have fused thereto other compounds which alter the polypeptides biological or pharmacological properties i.e. polyethylene glycol (PEG) to increase half-life; leader or secretory amino acid sequences for ease of purification; prepro- and pro- sequences; and (poly)saccharides.

Furthermore, in those situations where amino acid regions are found to be polymorphic, it may be desirable to vary one or more particular amino acids to more effectively mimic the different epitopes of the different streptococcus strains.

Moreover, the polypeptides of the present invention can be modified by terminal $-NH_2$ acylation (eg. by acetylation, or thioglycolic acid amidation, terminal carboxy amidation, e.g. with ammonia or methylamine) to provide stability, increased hydrophobicity for linking or binding to a support or other molecule.

Also contemplated are hetero and homo polypeptide multimers of the polypeptide fragments and analogues. These polymeric forms include, for example, one or more polypeptides that have been cross-linked with cross-linkers such as avidin/biotin, gluteraldehyde or dimethylsuperimidate. Such polymeric forms also include polypeptides containing two or more tandem or inverted contiguous sequences, produced from multicistronic mRNAs generated by recombinant DNA technology. In a further embodiment, the present invention also relates to chimeric polypeptides which comprise one or more polypeptides or fragments or analogs thereof as defined in the figures of the present application.

In a further embodiment, the present invention also relates to chimeric polypeptides comprising two or more polypeptides having a sequence chosen from SEQ ID NO: 2, or fragments or analogs thereof;

provided that the polypeptides are linked as to formed a chimeric polypeptide.

In a further embodiment, the present invention also relates to chimeric polypeptides comprising two or more polypeptides having a sequence chosen from SEQ ID NO: 2 provided that the polypeptides are linked as to formed a chimeric polypeptide.

Preferably, a fragment, analog or derivative of a polypeptide of the invention will comprise at least one antigenic region i.e. at least one epitope.

In order to achieve the formation of antigenic polymers (i.e. synthetic multimers), polypeptides may be utilized having bishaloacetyl groups, nitroarylhalides, or the like, where the reagents being specific for thio groups. Therefore, the link between two mercapto groups of the different polypeptides may be a single bond or may be composed of a linking group of at least two, typically at least four, and not more than 16, but usually not more than about 14 carbon atoms.

In a particular embodiment, polypeptide fragments and analogs of the invention do not contain a methionine (Met) starting residue. Preferably, polypeptides will not incorporate a leader or secretory sequence (signal sequence). The signal portion of a polypeptide of the invention may be determined according to established molecular biological techniques. In general, the polypeptide of interest may be isolated from a streptococcal culture and subsequently sequenced to determine the initial residue of the mature protein and therefore the sequence of the mature polypeptide.

It is understood that polypeptides can be produced and/or used without their start codon (methionine or valine) and/or without their leader peptide to favor production and purification of recombinant polypeptides. It is known that cloning genes without sequences encoding leader peptides will restrict the polypeptides to the cytoplasm of E. coli and will facilitate their recovery

(Glick, B.R. and Pasternak, J.J. (1998) Manipulation of gene expression in prokaryotes. In "Molecular biotechnology: Principles and applications of recombinant DNA", 2nd edition, ASM Press, Washington DC, p.109-143).

According to another aspect of the invention, there are also provided (i) a composition of matter containing a polypeptide of the invention, together with a carrier, diluent or adjuvant; (ii) a pharmaceutical composition comprising a polypeptide of the invention and a carrier, diluent or adjuvant; (iii) a vaccine comprising a polypeptide of the invention and a carrier, diluent or adjuvant; (iv) a method for inducing an immune response against Streptococcus, in a host, by administering to the host, an immunogenically effective amount of a polypeptide of the invention to elicit an immune response, e.g., a protective immune response to Streptococcus; and particularly, (v) a method for preventing and/or treating a Streptococcus infection, by administering a prophylactic or therapeutic amount of a polypeptide of the invention to a host in need.

According to another aspect of the invention, there are also provided (i) a composition of matter containing a polynucleotide of the invention, together with a carrier, diluent or adjuvant; (ii) a pharmaceutical composition comprising a polynucleotide of the invention and a carrier, diluent or adjuvant; (iii) a method for inducing an immune response against Streptococcus, in a host, by administering to the host, an immunogenically effective amount of a polynucleotide of the invention to elicit an immune response, e.g., a protective immune response to Streptococcus; and particularly, (iv) a method for preventing and/or treating a Streptococcus infection, by administering a prophylactic or therapeutic amount of a polynucleotide of the invention to a host in need.

Before immunization, the polypeptides of the invention can also be coupled or conjugated to carrier proteins such as tetanus toxin, diphtheria toxin, hepatitis B virus surface antigen, poliomyelitis virus VP1 antigen or any other viral or bacterial toxin or antigen

or any suitable proteins to stimulate the development of a stronger immune response. This coupling or conjugation can be done chemically or genetically. A more detailed description of peptide-carrier conjugation is available in Van Regenmortel, M.H.V., Briand J.P., Muller S., Plaué S., «Synthetic Polypeptides as antigens» in Laboratory Techniques in Biochemistry and Molecular Biology, Vol. 19 (ed.) Burdou, R.H. & Van Knippenberg P.H. (1988), Elsevier New York.

According to another aspect, there are provided pharmaceutical compositions comprising one or more Streptococcal polypeptides of the invention in a mixture with a pharmaceutically acceptable adjuvant. Suitable adjuvants include (1) oil-in-water emulsion formulations such as MF59™, SAF™, Ribi™ ; (2) Freund's complete or incomplete adjuvant; (3) salts i.e. $AlK(SO_4)_2$, $AlNa(SO_4)_2$, $AlNH_4(SO_4)_2$, $Al(OH)_3$, $AlPO_4$, silica, kaolin; (4) saponin derivatives such as Stimulon™ or particles generated therefrom such as ISCOMs (immunostimulating complexes); (5) cytokines such as interleukins, interferons, macrophage colony stimulating factor (M-CSF), tumor necrosis factor (TNF) ; (6) other substances such as carbon polynucleotides i.e. poly IC and poly AU, detoxified cholera toxin (CTB)and E.coli heat labile toxin for induction of mucosal immunity. A more detailed description of adjuvant is available in a review by M.Z.I Khan et al. in Pharmaceutical Research, vol. 11, No. 1 (1994) pp2-11, and also in another review by Gupta et al., in Vaccine, Vol. 13, No. 14, pp1263-1276 (1995) and in WO 99/24578, which are herein incorporated by reference. Preferred adjuvants include QuilA™, QS21™, Alhydrogel™ and Adjuphos™.

Pharmaceutical compositions of the invention may be administered parenterally by injection, rapid infusion, nasopharyngeal absorption, dermoabsorption, or buccal or oral.

Pharmaceutical compositions of the invention are used for the treatment or prophylaxis of streptococcal infection and/or diseases and symptoms mediated by streptococcal infection as described in P.R. Murray (Ed, in chief),E.J. Baron, M.A. Pfaller, F.C. Tenover

and R.H. Yolken. Manual of Clinical Microbiology, ASM Press, Washington, D.C. sixth edition, 1995, 1482p which are herein incorporated by reference. In one embodiment, pharmaceutical compositions of the present invention are used for the prophylaxis or treatment of pharyngitis, erysipelas and impetigo, scarlet fever, and invasive diseases such as bacteremia and necrotizing fasciitis and also toxic shock. In one embodiment, pharmaceutical compositions of the invention are used for the prophylaxis or treatment of Streptococcus infection and/or diseases and symptoms mediated by Streptococcus infection, in particular group A Streptococcus (Streptococcus pyogenes), group B Streptococcus (GBS or S.agalactiae), S.pneumoniae, S.dysgalactiae, S.uberis, S.nocardia as well as Staphylococcus aureus. In a further embodiment, the Streptococcus infection is S. pyogenes.

In a further embodiment, the invention provides a method for prophylaxis or treatment of Streptococcus infection in a host susceptible to Streptococcus infection comprising administering to said host a therapeutic or prophylactic amount of a composition of the invention.

As used in the present application, the term "host" includes mammals. In a further embodiment, the mammal is human.

In a particular embodiment, pharmaceutical compositions are administered to those hosts at risk of streptococcus infection such as infants, elderly and immunocompromised hosts.

Pharmaceutical compositions are preferably in unit dosage form of about 0.001 to 100 µg/kg (antigen/body weight) and more preferably 0.01 to 10 µg/kg and most preferably 0.1 to 1 µg/kg 1 to 3 times with an interval of about 1 to 6 week intervals between immunizations.

Pharmaceutical compositions are preferably in unit dosage form of about 0.1 µg to 10 mg and more preferably 1µg to 1 mg and most

preferably 10 to 100 µg 1 to 3 times with an interval of about 1 to 6 week intervals between immunizations.

According to another aspect, there are provided polynucleotides encoding polypeptides characterized by the amino acid sequence comprising SEQ ID NO: 2 or fragments or analogs thereof.

In one embodiment, polynucleotides are those illustrated in SEQ ID No: 1 which may include the open reading frames (ORF), encoding the polypeptides of the invention.

It will be appreciated that the polynucleotide sequences illustrated in the figures may be altered with degenerate codons yet still encode the polypeptides of the invention. Accordingly the present invention further provides polynucleotides which hybridize to the polynucleotide sequences herein above described (or the complement sequences thereof) having 50% identity between sequences. In one embodiment, at least 70% identity between sequences. In one embodiment, at least 75% identity between sequences. In one embodiment, at least 80% identity between sequences. In one embodiment, at least 85% identity between sequences. In one embodiment, at least 90% identity between sequences. In a further embodiment, polynucleotides are hybridizable under stringent conditions i.e. having at least 95% identity. In a further embodiment, more than 97% identity.

Suitable stringent conditions for hybridation can be readily determined by one of skilled in the art (see for example Sambrook et al., (1989) Molecular cloning : A Laboratory Manual, 2nd ed, Cold Spring Harbor, N.Y.; Current Protocols in Molecular Biology, (1999) Edited by Ausubel F.M. et al., John Wiley & Sons, Inc., N.Y.).

In a further embodiment, the present invention provides polynucleotides that hybridize under stringent conditions to either

(a) a DNA sequence encoding a polypeptide or

(b) the complement of a DNA sequence encoding a polypeptide; wherein said polypeptide comprises SEQ ID NO: 2, or fragments or analogs thereof.

In a further embodiment, the present invention provides polynucleotides that hybridize under stringent conditions to either

(a) a DNA sequence encoding a polypeptide or

(b) the complement of a DNA sequence encoding a polypeptide; wherein said polypeptide comprises SEQ ID NO: 2.

In a further embodiment, the present invention provides polynucleotides that hybridize under stringent conditions to either

(a) a DNA sequence encoding a polypeptide or

(b) the complement of a DNA sequence encoding a polypeptide; wherein said polypeptide comprises at least 10 contiguous amino acid residues from a polypeptide comprising SEQ ID NO: 2, or fragments or analogs thereof.

In a further embodiment, the present invention provides polynucleotides that hybridize under stringent conditions to either

(a) a DNA sequence encoding a polypeptide or

(b) the complement of a DNA sequence encoding a polypeptide; wherein said polypeptide comprises at least 10 contiguous amino acid residues from a polypeptide comprising SEQ ID NO: 2.

In a further embodiment, polynucleotides are those encoding polypeptides of the invention illustrated in SEQ ID NO: 2 or fragments or analogs thereof.

In a further embodiment, polynucleotides are those illustrated in SEQ ID NO: 1 encoding polypeptides of the invention or fragments or analogs thereof.

In a further embodiment, polynucleotides are those encoding polypeptides of the invention illustrated in SEQ ID NO: 2.

In a further embodiment, polynucleotides are those illustrated in SEQ ID NO: 1 encoding polypeptides of the invention.

As will be readily appreciated by one skilled in the art, polynucleotides include both DNA and RNA.

The present invention also includes polynucleotides complementary to the polynucleotides described in the present application.

In a further aspect, polynucleotides encoding polypeptides of the invention, or fragments, analogs or derivatives thereof, may be used in a DNA immunization method. That is, they can be incorporated into a vector which is replicable and expressible upon injection thereby producing the antigenic polypeptide in vivo. For example polynucleotides may be incorporated into a plasmid vector under the control of the CMV promoter which is functional in eukaryotic cells. Preferably the vector is injected intramuscularly.

According to another aspect, there is provided a process for producing polypeptides of the invention by recombinant techniques by expressing a polynucleotide encoding said polypeptide in a host cell and recovering the expressed polypeptide product. Alternatively, the polypeptides can be produced according to established synthetic chemical techniques i.e. solution phase or solid phase synthesis of oligopeptides which are ligated to produce the full polypeptide (block ligation).

General methods for obtention and evaluation of polynucleotides and polypeptides are described in the following references: Sambrook et al, Molecular Cloning: A Laboratory Manual, 2nd ed, Cold Spring Harbor, N.Y., 1989; Current Protocols in Molecular Biology, Edited by Ausubel F.M. et al., John Wiley and Sons, Inc. New York; PCR Cloning Protocols, from Molecular Cloning to Genetic Engineering,

Edited by White B.A., Humana Press, Totowa, New Jersey, 1997, 490 pages; Protein Purification, Principles and Practices, Scopes R.K., Springer-Verlag, New York, 3rd Edition, 1993, 380 pages; Current Protocols in Immunology, Edited by Coligan J.E. et al., John Wiley & Sons Inc., New York which are herein incorporated by reference.

For recombinant production, host cells are transfected with vectors which encode the polypeptide, and then cultured in a nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying the genes. Suitable vectors are those that are viable and replicable in the chosen host and include chromosomal, non-chromosomal and synthetic DNA sequences e.g. bacterial plasmids, phage DNA, baculovirus, yeast plasmids, vectors derived from combinations of plasmids and phage DNA. The polypeptide sequence may be incorporated in the vector at the appropriate site using restriction enzymes such that it is operably linked to an expression control region comprising a promoter, ribosome binding site (consensus region or Shine-Dalgarno sequence), and optionally an operator (control element). One can select individual components of the expression control region that are appropriate for a given host and vector according to established molecular biology principles (Sambrook et al, Molecular Cloning: A Laboratory Manual, 2nd ed, Cold Spring Harbor, N.Y., 1989; Current Protocols in Molecular Biology, Edited by Ausubel F.M. et al., John Wiley and Sons, Inc. New York incorporated herein by reference). Suitable promoters include but are not limited to LTR or SV40 promoter, E.coli lac, tac or trp promoters and the phage lambda P_L promoter. Vectors will preferably incorporate an origin of replication as well as selection markers i.e. ampicilin resistance gene. Suitable bacterial vectors include pET, pQE70, pQE60, pQE-9, pD10 phagescript, psiX174, pbluescript SK, pbsks, pNH8A, pNH16a, pNH18A, pNH46A, ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 and eukaryotic vectors pBlueBacIII, pWLNEO, pSV2CAT, pOG44, pXT1, pSG, pSVK3, pBPV, pMSG and pSVL. Host cells may be bacterial i.e. E.coli, Bacillus subtilis, Streptomyces; fungal i.e. Aspergillus niger, Aspergillus nidulins; yeast i.e. Saccharomyces or eukaryotic i.e. CHO, COS.

Upon expression of the polypeptide in culture, cells are typically harvested by centrifugation then disrupted by physical or chemical means (if the expressed polypeptide is not secreted into the media) and the resulting crude extract retained to isolate the polypeptide of interest. Purification of the polypeptide from culture media or lysate may be achieved by established techniques depending on the properties of the polypeptide i.e. using ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, hydroxylapatite chromatography and lectin chromatography. Final purification may be achieved using HPLC.

The polypeptides may be expressed with or without a leader or secretion sequence. In the former case the leader may be removed using post-translational processing (see US 4,431,739; US 4,425,437; and US 4,338,397 incorporated herein by reference) or be chemically removed subsequent to purifying the expressed polypeptide.

According to a further aspect, the streptococcal polypeptides of the invention may be used in a diagnostic test for Streptococcus infection, in particular S. pyogenes infection. Several diagnostic methods are possible, for example detecting Streptococcus organism in a biological sample, the following procedure may be followed:

a) obtaining a biological sample from a host;

b) incubating an antibody or fragment thereof reactive with a Streptococcus polypeptide of the invention with the biological sample to form a mixture; and

c) detecting specifically bound antibody or bound fragment in the mixture which indicates the presence of Streptococcus.

Alternatively, a method for the detection of antibody specific to a Streptococcus antigen in a biological sample containing or suspected of containing said antibody may be performed as follows:

a) obtaining a biological sample from a host;

b) incubating one or more Streptococcus polypeptides of the invention or fragments thereof with the biological sample to form a mixture; and

c) detecting specifically bound antigen or bound fragment in the mixture which indicates the presence of antibody specific to Streptococcus.

One of skill in the art will recognize that this diagnostic test may take several forms, including an immunological test such as an enzyme-linked immunosorbent assay (ELISA), a radioimmunoassay or a latex agglutination assay, essentially to determine whether antibodies specific for the protein are present in an organism.

The DNA sequences encoding polypeptides of the invention may also be used to design DNA probes for use in detecting the presence of Streptococcus in a biological sample suspected of containing such bacteria. The detection method of this invention comprises:

a) obtaining the biological sample from a host;

b) incubating one or more DNA probes having a DNA sequence encoding a polypeptide of the invention or fragments thereof with the biological sample to form a mixture; and

c) detecting specifically bound DNA probe in the mixture which indicates the presence of Streptococcus bacteria.

The DNA probes of this invention may also be used for detecting circulating Streptococcus i.e. S. pyogenes nucleic acids in a sample, for example using a polymerase chain reaction, as a method of diagnosing Streptococcus infections. The probe may be synthesized using conventional techniques and may be immobilized on a solid phase, or may be labelled with a detectable label. A preferred DNA probe for this application is an oligomer having a sequence complementary to at least about 6 contiguous nucleotides of the S. pyogenes polypeptides of the invention.

Another diagnostic method for the detection of Streptococcus in a host comprises:

a) labelling an antibody reactive with a polypeptide of the invention or fragment thereof with a detectable label;

b) administering the labelled antibody or labelled fragment to the host; and

c) detecting specifically bound labelled antibody or labelled fragment in the host which indicates the presence of Streptococcus.

According to one aspect, the present invention provides the use of an antibody for treatment and/or prophylaxis of streptococcal infections.

A further aspect of the invention is the use of the Streptococcus polypeptides of the invention as immunogens for the production of specific antibodies for the diagnosis and in particular the treatment of streptococcus infection. Suitable antibodies may be determined using appropriate screening methods, for example by measuring the ability of a particular antibody to passively protect against streptococcus infection in a test model. One example of an animal model is the mouse model described in the examples herein. The antibody may be a whole antibody or an antigen-binding fragment thereof and may belong to any immunoglobulin class. The antibody or fragment may be of animal origin, specifically of mammalian origin and more specifically of murine, rat or human origin. It may be a natural antibody or a fragment thereof, or if desired, a recombinant antibody or antibody fragment. The term recombinant antibody or antibody fragment means antibody or antibody fragment which was produced using molecular biology techniques. The antibody or antibody fragments may be polyclonal, or preferably monoclonal. It may be specific for a number of epitopes associated with the S. pyogenes polypeptides but is preferably specific for one.

A further aspect of the invention is the use of the antibodies directed to the polypeptides of the invention for passive immunization. One could use the antibodies described in the present application.

A further aspect of the invention is a method for immunization, whereby an antibody raised by a polypeptide of the invention is administered to a host in an amount sufficient to provide a passive immunization.

In a further embodiment, the invention provides the use of a pharmaceutical composition in the manufacture of a medicament for the prophylactic or therapeutic treatment of streptococcal infection.

In a further embodiment, the invention provides a kit comprising a polypeptide of the invention for detection or diagnosis of streptococcal infection.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

EXAMPLE 1

This example illustrates the cloning and molecular characteristics of BVH-P7 gene and corresponding polypeptide.

The coding region of S. pyogenes BVH-P7 (SEQ ID NO: 1) gene was amplified by PCR (Robocycler Gradient 96 Temperature cycler, Stratagene, LaJolla, CA) from genomic DNA of serotype M1 S. pyogenes strain ATCC700294 using the following oligonuceotide primers that contained base extensions for the addition of restriction sites *Nde*I (CATATG) and *Not*I (GCGGCCGC): DMAR293 and DMAR294, which are presented in Table 1. PCR products were purified from agarose gel using a QIAquick gel extraction kit from QIAgen following the manufacturer's instructions (Chatsworth, CA), and

digested with *Nde*I and *Not*I (Amersham Pharmacia Biotech Inc, Baie d'Urfé, Canada). The pET-21b(+) vector (Novagen, Madison, WI) was digested with *Nde*I and *Not*I and purified from agarose gel using a QIAquick gel extraction kit from QIAgen (Chatsworth, CA). The *Nde*I-*Not*I PCR products were ligated to the *Nde*I-*Not*I pET-21b(+) expression vector. The ligated products were transformed into E. coli strain DH5• [ϕ80d*lac*ZΔM15 Δ(*lac*ZYA-*arg*F)U169 *end*A1 *rec*A1 *hsd*R17(r_K^--m_K+) *deo*R *thi*-1 *sup*E44 λ⁻ *gyr*A96 *rel*A1] (Gibco BRL, Gaithersburg, MD) according to the method of Simanis (Hanahan, D. DNA Cloning, 1985, D.M. Glover (ed), pp. 109-135). Recombinant pET-21b(+) plasmid (rpET21b(+)) containing BVH-P7 gene was purified using a QIAgen plasmid kit (Chatsworth, CA) and DNA insert was sequenced (Taq Dye Deoxy Terminator Cycle Sequencing kit, ABI, Foster City, CA).

Table 1. Oligonucleotide primers used for PCR amplifications of S. pyogenes BVH-P7 gene

Genes	Primers I.D. (SEQ ID NO)	Restriction site	Vector	Sequence
BVH-P7	DMAR293 (3)	*NdeI*	pET21b	5'-GTAGTCACCCACCATATGGAAGTTTTTAG-3'
BVH-P7	DMAR294 (4)	*NotI*	pET21b	5'-TTTTTTCTTTGCGGCCGCAGTTATTAGT-3'
BVH-P7	DMAR480a (5)	*BamHI*	pCMV-GH	5'-GGGGATCCCACCCACAATCAGG-3'
BVH-P7	DMAR481a (6)	*SalI*	pCMV-GH	5'-GGTTGTCGACAGTAAAGCAACGCTAGTG-3'

It was determined that the 3027-bp including a stop codon (TAA) open reading frame (ORF) of BVH-P7 encodes a 1008 amino-acid-residues polypeptide with a predicted pI of 6.18 and a predicted molecular mass of 111,494.44 Da. Analysis of the predicted amino acid residues sequence (SEQ ID NO :2) using the PSORTII sofware (Real World Computing Partnership (http://psort.nibb.ac.jp)) suggested the existence of a 21 amino acid residues signal peptide (MKKHLKTVALTLTTVSVVTHN), which ends with a cleavage site situated between an asparagine and a glutamine residues. Analysis of the amino-acid-residues sequence revealed the presence of a cell wall anchoring motif (LPXTGX) located bewteen residues 974 and 981.

To confirm the presence by PCR amplification of BVH-P7 (SEQ ID NO :1) gene, the following 4 serologically distinct S. pyogenes strains were used: the serotype M1 S. pyogenes strain ATCC700294 and the serotype M3 S. pyogenes strain ATCC12384 were obtained from the American Type Culture Collection (Rockville, MD); the serotype M6 S. pyogenes SPY67 clinical isolate was provided by the Centre de recherche en infectiologie du Centre hospitalier de l'université Laval, Sainte-Foy; and S. pyogenes strain B514 which was initially

isolated from a mouse was provided by Susan Hollingshead, from University of Alabama, Birmingham. The E. coli strain XL1-Blue MRF' was used in these experiments as negative control. Chromosomal DNA was isolated from each S. pyogenes strain as previously described (Jayarao BM et al. 1991. J. Clin. Microbiol. 29:2774-2778). BVH-P7 (SEQ ID NO :1) gene was amplified by PCR (Robocycler Gradient 96 Temperature cycler, Stratagene, LaJolla, Ca) from the genomic DNA purified from the 4 S. pyogenes strains, and the control E. coli strain using the oligonucleotide primers DMAR293 and DMAR294 (Table 1). PCR was performed with 30 cycles of 45 sec at 95°C, 45 sec at 50°C and 2 min at 72°C and a final elongation period of 7 min at 72°C. The PCR products were size fractionated in 1% agarose gels and were visualized by ethidium bromide staining. The results of these PCR amplifications are presented in Table 2. The analysis of the amplification products revealed that BVH-P7 (SEQ ID NO :1) gene was present in the genome of all of the 4 S. pyogenes strains tested. No such product was detected when the control E. coli DNA was submitted to identical PCR amplifications with these oligonucleotide primers.

Table 2. Identification of S. pyogenes BVH-P7 gene by PCR amplification in the genome of four serologically distinct S. pyogenes strains

Strain Identification	Identification of BVH-P7 gene
ATCC700294 (M1)	+
ATCC12384 (M3)	+
SPY67 (M6)	+
B514*	+
E. coli XL1 Blue MRF'	-

*Mouse isolate

EXAMPLE 2

This example illustrates the cloning of S. pyogenes BVH-P7 gene in CMV plasmid pCMV-GH.

The DNA coding region of S. pyogenes protein was inserted in phase downstream of a human growth hormone (hGH) gene which was under the transcriptional control of the cytomegalovirus (CMV) promotor in the plasmid vector pCMV-GH (Tang et al., Nature, 1992, 356 :152). The CMV promotor is a non functional plasmid in E. coli cells but active upon administration of the plasmid in eukaryotic cells. The vector also incorporated the ampicillin resistance gene.

The coding regions of BVH-P7 (SEQ ID NO: 1) gene without its leader peptide region was amplified by PCR (Robocycler Gradient 96 Temperature cycler, Stratagene, LaJolla, CA) from genomic DNA of serotype M1 S. pyogenes strain ATCC700294 using oligonucleotide primers DMAR480a and DMAR481a that contained base extensions for the addition of restriction sites *Bam*HI (GGATCC) and *Sal*I (GTCGAC) which are described in Table 1. The PCR products were purified from agarose gel using a QIAquick gel extraction kit from QIAgen (Chatsworth, CA), digested with restriction enzymes (Amersham Pharmacia Biotech Inc, Baie d'Urfé, Canada). The pCMV-GH vector (Laboratory of Dr. Stephen A. Johnston, Department of Biochemistry, The University of Texas, Dallas, Texas) was digested with *Bam*HI and *Sal*I and purified from agarose gel using the QIAquick gel extraction kit from QIAgen (Chatsworth, CA). The *Bam*HI-*Sal*I DNA fragment was ligated to the *Bam*HI-*Sal*I-pCMV-GH vector to create the hGH-BVH-P7 fusion protein under the control of the CMV promoter. The ligated product was transformed into E. coli strain DH5• [ϕ80d*lac*ZΔM15 Δ(*lac*ZYA-*arg*F)U169 *end*A1 *rec*A1 *hsd*R17(r_K-m_K+) *deo*R *thi*-1 *sup*E44 λ⁻ *gyr*A96 *rel*A1] (Gibco BRL, Gaithersburg, MD) according to the method of Simanis (Hanahan, D. DNA Cloning, 1985, D.M. Glover (ed), pp. 109-135). The recombinant pCMV plasmid was purified using a QIAgen plasmid kit (Chatsworth, CA) and the nucleotide sequence of the DNA insert was verified by DNA sequencing.

EXAMPLE 3

This example illustrates the use of DNA to elicit an immune response to S. pyogenes BVH-P7 protein antigen.

Groups of 8 female BALB/c mice (Charles River, St-Constant, Québec, Canada) were immunized by intramuscular injection of 100 µl three times at two- or three-week intervals with 50 µg of recombinant pCMV-GH encoding BVH-P7 (SEQ ID NO: 1) gene in presence of 50 µg of granulocyte-macrophage colony-stimulating factor (GM-CSF)-expressing plasmid pCMV-GH-GM-CSF (Laboratory of Dr. Stephen A. Johnston, Department of Biochemistry, The University of Texas, Dallas, Texas). As control, groups of mice were injected with 50 µg of pCMV-GH in presence of 50 µg of pCMV-GH-GM-CSF. Blood samples were collected from the orbital sinus prior to each immunization and seven days following the third injection and serum antibody responses were determined by ELISA using the BVH-P7 His-tagged labeled S. pyogenes recombinant protein as coating antigen. The production and purification of this BVH-P7 His-tagged labeled S. pyogenes recombinant protein is presented in Example 4.

EXAMPLE 4

This example illustrates the production and purification of S. pyogenes BVH-P7 recombinant protein.

The recombinant pET-21b(+)plasmid with BVH-P7 (SEQ ID NO: 1) gene was used to transform by electroporation (Gene Pulser II apparatus, BIO-RAD Labs, Mississauga, Canada) E. coli strain Tuner (DE3) (F^- ompT $hsdS_B$ ($r^-_B m^-_B$) gal dcm lacYI (DE3)) (Novagen, Madison, WI). In this strain of E. coli, the T7 promotor controlling expression of the recombinant protein is specifically recognized by the T7 RNA polymerase (present on the λDE3 prophage) whose gene is under the control of the lac promotor which is inducible by isopropyl-ß-d-thio-galactopyranoside (IPTG). The transformants Tuner (DE3)/rpET21 (+) were grown at 37°C with agitation at 250 rpm in LB broth (peptone 10g/L, yeast extract 5g/L, NaCl 10g/L) containing 100 µg of carbenicillin (Sigma-Aldrich Canada Ltd., Oakville, Canada) per ml until the A_{600} reached a value of 0.6. In order to induce the production of BVH-P7 His-tagged S. pyogenes recombinant protein, the cells were incubated for 3 additional hours in the

presence of IPTG at a final concentration of 0.1 mM. Induced cells from a 500 ml culture were pelleted by centrifugation and frozen at -70°C.

The purification of the BVH-P7 His-tagged recombinant protein from the non-soluble fraction of IPTG-induced Tuner (DE3)/rpET21b(+) was done by affinity chromatography based on the properties of the His•Tag sequence (6 consecutive histidine residues) to bind to divalent cations (Ni^{2+}) immobilized on the His•Bind metal chelation resin. Briefly, the pelleted cells obtained from a 500 mL culture induced with IPTG was resuspended in lysis buffer (20 mM Tris, 500 mM NaCl, 10 mM imidazole, pH 7.9) containing 6M Guanidine-HCl, sonicated and centrifuged at 12,000 X g for 20 min to remove debris. The supernatant was incubated with Ni-NTA agarose resin (Qiagen, Mississauga, Ontario, Canada) for 45 min at 4°C. The BVH-P7 His-tagged S. pyogenes recombinant protein was eluted from the resin with a solution containing 6M Guanidine-HCl and 250 mM imidazole-500mM NaCl-20 mM Tris, pH 7.9. The removal of the salt and imidazole from the samples was done by dialysis against 10mM Tris and 0.9% NaCl, pH 7.9 overnight at 4°C. The amount of recombinant protein was estimated by MicroBCA (Pierce, Rockford, Illinois).

EXAMPLE 5

This example illustrates the reactivity of the BVH-P7 His-tagged S. pyogenes recombinant protein with human sera and sera collected from mice after immunization with S. pyogenes antigenic preparations.

As shown in Table 3, purified His-tagged BVH-P7 recombinant protein was recognized in immunoblots by the antibodies present in the pool of normal sera. This is an important result since it clearly indicates that human which are normally in contact with S. pyogenes do develop antibodies that are specific to that protein. These particular human antibodies might be implicated in the protection against S. pyogenes infection. In addition, immunoblots also

revealed that sera collected from mice immunized with S. pyogenes antigenic preparations enriched membrane proteins which protected mice against lethal challenge also developed antibodies that recognized BVH-P7 His-tagged recombinant protein. This result indicates that this protein was present in S. pyogenes antigenic preparation that protected mice against infection and that this streptococcal protein induced antibodies that reacted with the corresponding His-tagged recombinant protein.

Table 3. Reactivity in immunoblots of human sera and sera collected from mice after immunization with S. pyogenes antigenic preparations with BVH-P7 His-tagged recombinant protein.

Purified recombinant protein I.D.[1]	**Apparent molecular weight (kDa)**[2]	**Reactivity in immunoblots with**	
		Human sera[3]	Mouse sera[4]
BVH-P7	110	+	+

[1]BVH-P7 His-tagged recombinant protein produced and purified as described in Example 7 was used to perform the immunoblots.

[2]Molecular weight of the BVH-P7 His-tagged recombinant protein was estimated after SDS-PAGE.

[3]Two sera collected from healthy human volunteers were pooled together and diluted 1/500 to perform the immunoblots.

[4]Mouse sera collected after immunization with S. pyogenes antigenic preparations enriched membrane proteins were pooled and diluted 1/500 to perform the immunoblots. These mice were protected against a lethal S. pyogenes challenge.

EXAMPLE 6

This example illustrates the accessibility to antibodies of the S. pyogenes BVH-P7 protein at the surface of intact streptococcal cells.

Bacteria were grown in Tood Hewitt (TH) broth (Difco Laboratories, Detroit, MI) with 0.5% Yeast extract (Difco Laboratories) and 0.5%

peptone extract (Merck, Darmstadt, Germany) at 37°C in a 8% CO_2 atmosphere to give an OD_{490nm} of 0.600 (~10^8 CFU/ml). Dilutions of anti-BVH-P7 or control sera were then added and allowed to bind to the cells, which were incubated for 2 h at 4°C. Samples were washed 4 times in blocking buffer [phosphate-buffered saline (PBS) containing 2% bovine serum albumin (BSA)], and then 1 ml of goat fluorescein (FITC)-conjugated anti-mouse IgG + IgM diluted in blocking buffer was added. After an additional incubation of 60 min at room temperature, samples were washed 4 times in blocking buffer and fixed with 0.25 % formaldehyde in PBS buffer for 18-24 h at 4°C. Cells were washed 2 times in PBS buffer and resuspended in 500 µl of PBS buffer. Cells were kept in the dark at 4°C until analyzed by flow cytometry (Epics® XL; Beckman Coulter, Inc.). Ten thousands intact S. pyogenes cells were analyzed per sample and the results were expressed as percentage of labeled cells and fluorescence index. The fluorescence index was calculated as the median fluorescence value obtained after labeling the streptococcal cells with an immune serum divided by the fluorescence value obtained for a control mouse serum. A fluorescence value of 1 indicated that there was no binding of antibodies at the surface of intact streptococcal cells.

Sera collected from eight mice immunized with BVH-P7 His-tagged recombinant protein were analyzed by cytofluorometry and the results are presented in Table 4. All of the sera collected from mice immunized with purified BVH-P7 His-tagged protein contained BVH-P7-specific antibodies that efficiently recognized their corresponding surface exposed epitopes on the heterologous (ATCC12384; serotype M3) S. pyogenes strain tested. The fluorescence index varied from 10 to 18. It was determined that more than 97 % of the 10,000 S. pyogenes cells analyzed were labeled with the antibodies present in the BVH-P7 specific anti-sera. These sera were also pooled and reacted with the following S. pyogenes strains: serotype M1 S. pyogenes strain ATCC 700294, serotype M3 and serotype M18 S. pyogenes strain ATCC12357 were obtained from the American Type Culture Collection (Rockville, MD,

USA); the serotype M6 S. pyogenes SPY69 and M2 S. pyogenes SPY68 clinical isolates were provided by the Centre de recherche en infectiologie du Centre hospitalier de l'université Laval, Sainte-Foy. The BVH-P7-specific antibodies present in the pool of sera collected after immunization with the purified His-tagged recombinant BVH-P7 protein attached at the bacterial surface of each of these streptococcal strains with fluorescence index between 4 up to 9. On the contrary, no labeling of the streptococcal cells were noted when pools of sera collected from unimmunized or sham-immunized mice were used. These observations clearly demonstrate that the BVH-P7 protein is accessible at the surface where it can be easily recognized by antibodies. Anti-S. pyogenes antibodies were shown to play an important role in the protection against S. pyogenes infection.

Table 4. Evaluation of the attachment of BVH-P7-specific antibodies at the surface of intact cells of S. pyogenes ATCC12384 strain (serotype M3).

Serum Identification	Fluorescence Index[2]	% of labeled cells[3]
S1[1]	11	97
S2	11	97
S3	13	98
S4	16	99
S5	10	97
S6	12	97
S7	13	98
S8	18	99
Pool of negative control sera[4]	1	9
Positive control serum[5]	12	98

[1] The mice S1 to S8 were injected subcutaneously three times at three-week intervals with 20 μg of purified BVH-P7 recombinant protein mixed with 10 μg of QuilA adjuvant (Cedarlane Laboratories, Hornby, Canada). The sera were diluted 1/50.

[2] The fluorescence index was calculated as the median fluorescence value obtained after labeling the streptococcal cells with an immune serum divided by the fluorescence value obtained for a control mouse serum. A fluorescence value of 1 indicated that there was no binding of antibodies at the surface of intact streptococcal cells.

[3]% of streptococcal labeled cells out of the 10,000 cells analyzed.

[4] Sera collected from unimmunized or sham-immunized mice were pooled diluted 1/50 and used as negative controls for this assay.

[5]Serum obtained from a mouse immunized with 20 μg of purified streptococcal recombinant M protein, a well known surface protein, was diluted 1/200 and was used as a positive control for the assay.

EXAMPLE 7

This example illustrates the protection against fatal S. pyogenes infection induced by passive immunization of mice with rabbit hyper-immune sera.

New Zealand rabbits (Charles River laboratories, St-Constant, Canada) were injected subcutaneously at multiple sites with 50 μg and 100 μg of the BVH-P7 His-tagged recombinant protein that was produced and purified as described in Example 4 and adsorbed to Alhydrogel adjuvant (Superfos Biosector a/s). Rabbits were immunized three times at three-week intervals with the BVH-P7 His-tagged recombinant protein. Blood samples were collected three weeks after the third injection. The antibodies present in the serum were purified by precipitation using 40% saturated ammonium sulfate. Groups of 10 female CD-1 mice (Charles River) were injected intravenously with 500 μl of purified serum collected from rabbits immunized with the BVH-P7 His-tagged recombinant protein, or rabbits immunized with an unrelated control recombinant protein. Eighteen hours later the mice were challenged with approximately $2x10^7$ CFU of the type 3 S. pyogenes strain ATCC12384. Samples of the S. pyogenes challenge inoculum were plated on blood agar plates to determine the CFU and to verify the challenge dose. Deaths were recorded for a period of 5 days.

EXAMPLE 8

This example illustrates the protection of mice against fatal S. pyogenes infection induced by immunization with purified recombinant BVH-P7 protein.

Groups of 8 female Balb/c mice (Charles River, St-Constant, Québec, Canada) were immunized subcutaneously three times at two-week intervals with 20 μg of affinity purified BVH-P7 His-tagged recombinant protein in presence of 10 μg of QuilA adjuvant (Cedarlane Laboratories Ltd, Hornby, Canada) or, as control, with QuilA adjuvant alone in PBS. Blood samples were collected from the orbital sinus on day 1, 14 and 28 prior to each immunization and two weeks (day 42) following the third injection. One week later the mice were challenged with approximately $3x10^6$ CFU of the type 3 S. pyogenes strain ATCC 12384. Samples of the S. pyogenes challenge inoculum were plated on blood agar plates to determine the CFU and to verify the challenge dose. Deaths were recorded for a period of 7 days. Four of eight mice immunized with purified recombinant BVH-P7 protein were protected against the lethal challenge, compared to only 12 % (1/8) of mice which received the adjuvant alone (Table 1).

Table 5. Ability of recombinant BVH-P7 protein to elicit protection against GAS strain ATCC 12384 (Type 3).

Immunogen	No. mice surviving	% survival
20 μg BVH-P7 + 10% QuilA	4/8	50
QuilA adjuvant alone in PBS	1/8	12